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Research Article

Life table parameters and biological characteristics of citrus butterfly *Papilio demoleus* (Lepidoptera: Papilionidae) on various citrus hosts

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Abstract: Citrus swallow tail butterfly, *Papilio demoleus* L. is a pest of citrus in many parts of the world. We examined the effect of four different citrus hosts including *Citrus sinensis* (L.) (Tarocco and Valencia) *Citrus limon* (L.) (Eureka), and *Citrus paradisi* Macfad. (Shambarg) on its life table parameters and biological characteristics at 24 ± 1 °C, 75 ± 5% RH and a photoperiod of 16: 8 h (L: D). The developmental time of immature stages was longest on *C. sinensis* (Tarocco) (12.3 ± SE days). The intrinsic rate of natural increase was significantly affected by various citrus, being the highest on *C. sinensis* (Tarocco) (0.099 day^{-1}) and lowest on *C. sinensis* (Valencia) (0.011 day^{-1}). The highest and lowest finite rates of increase were observed on *C. sinensis* (Tarocco) 1.10 and *C. sinensis* (Valencia) 1.01, respectively. The highest gross reproductive rate and the lowest mean generation time were observed on *C. sinensis* (Tarocco) and *C. sinensis.* (Valencia), respectively. The findings indicated that *C. sinensis* (Tarocco) appeared to be the most susceptible host to *P. demoleus* among the tested varieties. The results of this and similar studies in the future on other hosts may be considered as a basis for finding resistant host/hosts in monitoring this pest.

Keywords: *Papilio demoleus,* Life table, Population growth parameters, Citrus

Introduction

Citrus swallow tail butterfly, *Papilio demoleus* L. (Lepidoptera: Papilionidae), is an economically important pest of citrus worldwide (Homoziak *et al.*, 2006; Rajanikumar *et al.*, 2014). The caterpillars feed voraciously and cause severe damages to nurseries, young seedlings, and also they are capable of defoliating the trees, as a result, this pest is a potential threat to the citrus orchards (Larsen, 1984).

Host plant resistance is a part of the integrated pest management (IPM) and is relatively stable, cheap, non-polluting, and compatible with other methods of pest control (Sachan, 1990; Jallow *et al.*, 2004). Studying the different biological, physiological and ecological characteristics of a pest on different hosts is considered very important in finding a resistant host (Sarfraz *et al.*, 2007).

The study of feeding behavior and the effect of food quality on the biology of insects are important in recognizing suitable hosts (Greenberg *et al.*, 2001). The nutritional value, secondary metabolites and architectural characteristics of plants can all affect development, survival, fecundity, population growth and life table of herbivorous insects (Slansky and Feeney, 1977; Norris and Kogan, 1980; Montllor, 1991; Morgan *et al.*, 2001; Kim and Lee, 2002; Rutledge and O'Neil, 2005; Yasar and Gungor, 2005; Kumar *et al.*, 2009). Life table

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is considered as an integrated and comprehensive aspect of insect biology. It describes in details the developmental times, survival rates of each growth stage, fecundity and life expectancy of a population which could be regarded as essential tool in both theoretical and applied ecology (Chi, 1990; Carey, 1993; Taghizadeh *et al*., 2008). Population growth rate is a basic ecological characteristic that is usually described as the intrinsic rate of natural increase (r_m), an estimate of population growth potential was introduced by Birch (1948). The r_m is a key demographic parameter used to evaluate the level of plant resistance to insects. Southwood (1966) stated that the intrinsic rate of increase is the most practical life table parameter to compare the population growth potential of different species under specific climatic and food conditions and may help predict the outcomes of pest–natural enemy interactions. The r_m summarizes the physiological qualities of an animal relative to its capacity to increase (Andrewartha and Birch, 1954). Besides being a measure of population growth, r has been widely used as a bioclimatic index (Hulting *et al*., 1990), in estimation of insect response to resistant plants (Ruggle and Gutierrez, 1995), and in comparison of different food types that predators consumed (Engel, 1990). Although a large number of factors affect the components of r, there is a close and positive association between the mean relative growth rate and r (Guldemond *et al*., 1998). Pests usually cause more damage on susceptible host plants by having a higher population growth rate than on resistant ones. For this reason, having a basic knowledge on the population of a pest on different varieties of a host plant may provide an idea about the resistance or susceptibility of a particular host.

There is no information yet available on biology or life history of *P. demoleus* in Iran. Therefore, we undertook the present research intending to throw some light on the lack of knowledge on this pest.

Materials and Methods

Insect rearing and plant material

Four host plants were used in this study, including *Citrus sinensis* (Tarocco), *C. limon* (Eureka), *C. sinensis* (Valencia) and *C. paradisi* (Shambarg). Leaves of host plants of the mentioned species (on which rearing took place) were collected from citrus orchards near the city of Rudsar (37°08′15″N 50°17′17″E), north of Iran, Guilan province in 2016. The larvae were reared on fresh leaves of citrus varieties in the laboratory in growth chamber under controlled conditions at 24 ± 1 °C, 75 ± 5% RH and 16: 8 (L: D) h photoperiod in transparent plastic boxes (18 × 15 × 7cm) covered with muslin cloth for aeration. For adult hatching, the jar containing the larvae that pupated in were moved to containers of 18 × 7 dimensions. When adults emerged they were separated and placed in transparent cages (80 × 80 × 80 cm). Inside the cages, cotton soaked in 10% honey solution were provided for feeding of adults and small citrus seedlings for their oviposition. Eggs on the surface of leaves were separated and transferred to Petri dishes capped with a piece of humid cotton wool and placed in growth chamber in similar condition as above.

Growth and mortality of immature stages

This experiment was initiated with fifty eggs of *P. demoleus* maintained in Petri dishes inside the growth chamber. The hatching of eggs was carefully observed and recorded daily. Newly hatched larvae were transferred individually into a plastic box (5 × 6 × 12 cm) and were provided with fresh citrus leaves of each variety. The petioles of citrus leaves were wrapped in humid cotton wool pieces in order to provide humidity to the leaves. The leaves were changed every two days for earlier instars and daily for older larval instars. Larval growth was observed daily and the number of larval instars was determined based on the head capsules left after each stage. Developmental stages and mortality were observed and recorded daily.

Adult longevity and fecundity

One mating pair of butterfly on each variety (a total of 5 pairs for each variety) was transferred to a transparent cage (35 × 35 × 60 cm). A cotton wool soaked in 10% honey solution was provided for their feeding and a small citrus seedling for oviposition. Pre-oviposition, oviposition and post-oviposition periods were recorded. The oviposition cages were observed daily and the number of dead insects was recorded. In case of early female mortality, the male was provided with 10% honey and maintained in cage until death. The oviposition substrate and the cotton wool soaked in 10% honey were daily changed. The numbers of laid eggs were recorded daily. Observations continued until the death of the adults.

Statistical analysis

Data obtained from above experiments were used to calculate the life history and fertility parameters. The life history data were analyzed based on the age-stage and a two-sex life table analysis developed by Chi and Liu (1985). The age-stage specific survival rate (S_{xj}) (where x = age and j = stage), the age-specific survival rate (l_x), the age-specific fecundity (m_x), and the population parameters (r, the intrinsic rate of natural increase; λ, the finite rate of increase; R_0, the net reproductive rate; T, the mean generation time) were calculated accordingly. Data analysis and population parameters were calculated using the TWOSEX-MSChart program designed in visual BASIC for the Windows operation system (Chi, 2015). The TWOSEX-MSChart is available at http:// 140.120.197.173/Ecology/ prod02.htm (Chung Hsing University) and http://nhsbig.inhs.uiuc.edu /wes/chi.html (Illinois Natural History Survey). The standard errors of the developmental time, mean number of eggs per female, longevity and life table parameters were estimated by using 100,000 bootstraps. We used the paired bootstrap test to compare the differences between treatments based on the confidence interval of the difference between treatments (Efron and Tibshirani, 1993; Chi, 2015).

Results

Developmental times and survival rate

The developmental period of butterflies differed but no changes in the incubation period were observed among tested hosts (Table 1). As shown in Table 1, developmental time (egg to adult) of *P. demoleus* was the shortest on *C. sinensis* (Valencia) (9.67 ± 1.51) and the longest on *C. sinensis* (Tarocco) (12.38 ± 0.39).

Table 1 Development (Days, Mean ± SE) of various stages of *Papilio demoleus* on various citrus hosts at 24 ± 1 °C, 75 ± 5% RH and 16:8 (L: D) h.

Stage	*Citrus limon* (Eureka)	*Citrus paradisi* (Shambarg)	*Citrus sinensis* (Valencia)	*Citrus sinensis* (Tarocco)
Incubation period	4.00 ± 0.00^{a}	4.00 ± 0.00^{a}	4.00 ± 0.00^{a}	3.98 ± 0.02^{a}
First instar larvae	2.72 ± 0.11^{a}	2.72 ± 0.09^{ab}	3.02 ± 0.12^{a}	2.14 ± 0.05^{b}
Second instar larvae	2.37 ± 0.13^{b}	2.26 ± 0.13^{bc}	2.94 ± 0.10^{a}	2.04 ± 0.04^{c}
Third instar larvae	2.78 ± 0.10^{a}	2.77 ± 0.10^{a}	2.87 ± 0.11^{a}	2.22 ± 0.10^{b}
Fourth instar larvae	3.39 ± 0.12^{c}	3.61 ± 0.10^{bc}	3.78 ± 0.11^{b}	4.43 ± 0.10^{a}
Fifth instar larvae	7.6 ± 0.29^{a}	7.39 ± 0.26^{a}	6.38 ± 0.25^{b}	7.13 ± 0.18^{a}
Prepupae	1.60 ± 0.08^{a}	1.55 ± 0.10^{ab}	1.24 ± 0.10^{b}	1.72 ± 0.07^{a}
Pupae	13.70 ± 0.09^{b}	14.18 ± 0.07^{a}	13.15 ± 0.10^{c}	13.69 ± 0.07^{b}
Adult (male)	14.58 ± 0.29^{a}	14.91 ± 0.13^{a}	13 ± 0.13^{b}	15.05 ± 0.19^{a}
Adult (female)	9.95 ± 0.53^{a}	10.31 ± 0.64^{a}	8.00 ± 1.62^{b}	10.56 ± 0.30^{a}
Total (male & female)	12.09 ± 0.49^{a}	12.29 ± 0.57^{a}	9.67 ± 1.51^{b}	12.38 ± 0.39^{a}

Means in a row followed by the same letters are not significantly different at the 0.05 level (ANOVA).

Age-stage specific survival rate (S_{xj}) of *P. demoleus* on four citrus varieties is shown in Fig. 1. Age-stage-specific life expectancy (e_{ij}) represents the time that an individual of age i and stage j is expected to live (Fig. 2). Life expectancy for a newly laid Citrus swallow tail butterfly egg was 42, 33, 19 and 44 days on *C. limon* (Eureka), *C. paradisi* (Shambarg), *C. sinensis* (Valencia) and *C. sinensis* (Tarocco), respectively which slowly declined in all varieties. The life expectancy of adult males was more than that of adult females in all varieties (Fig. 2). Age-specific survival rate (l_x), age-stage specific fecundity of females (f_x), age-specific fecundity (m_x) and age-stage maternity (l_xm_x) of *P. demoleus* are plotted in Fig. 3. The age-specific survival rate represents the probability of survival of newly born individuals until age i. By combining all individuals, both sexes were considered. The curve of m_x is drawn on the basis of age after birth. The survival rate (l_x) was highest at the beginning and declined as age increased. The reproductive value (v_{xj}) is the expectation of future offspring of individuals of age x and stage j. The reproductive values for *P. demoleus* reared on *C. paradisi* (Shambarg) and *C. sinensis* (Tarocco) were higher than other varieties. Reproductive rate of *P. demoleus* reared on *C. paradisi* (Shambarg) and *C. sinensis* (Tarocco) reached its peak on day 38 and that of butterflies on *C. sinensis.* var. *valencia* and *C. limon* on days 48 and 40 (Fig. 4).

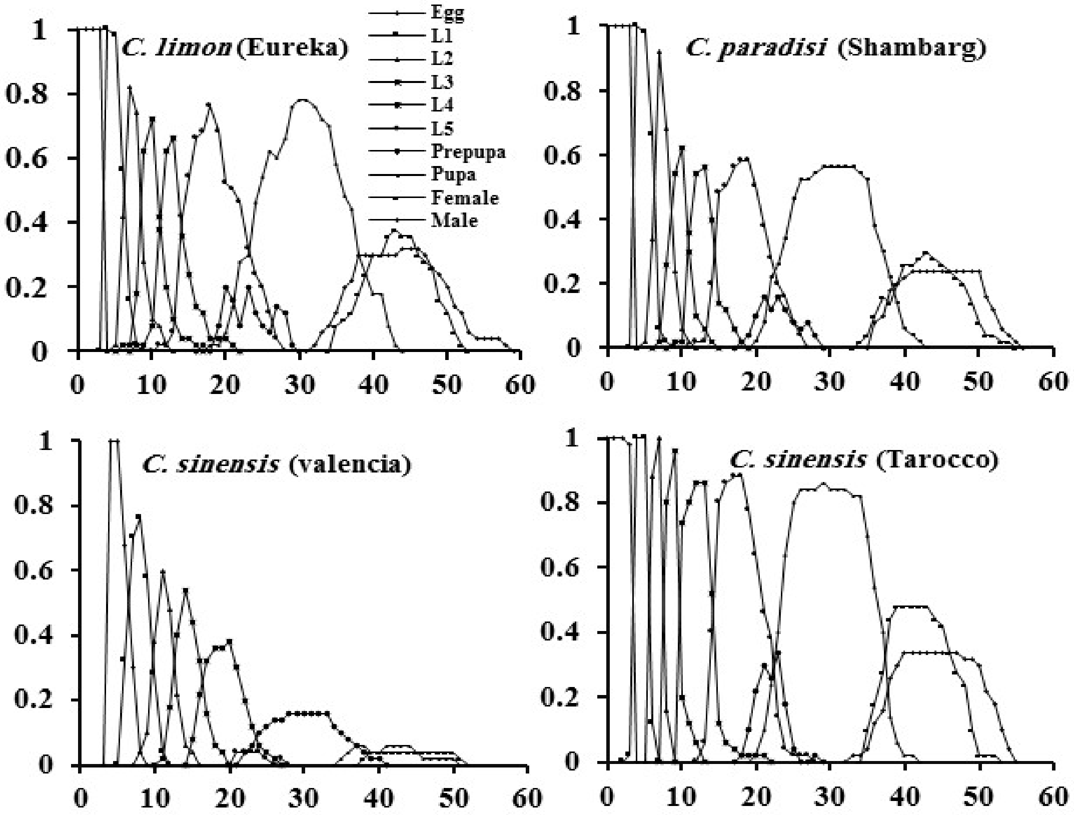

Figure 1 Age-stage survival rate (s_{xj}) of *Papilio demoleus* on various citrus hosts at 24 ± 1 °C, 75 ± 5% RH and 16: 8 (L: D) h.

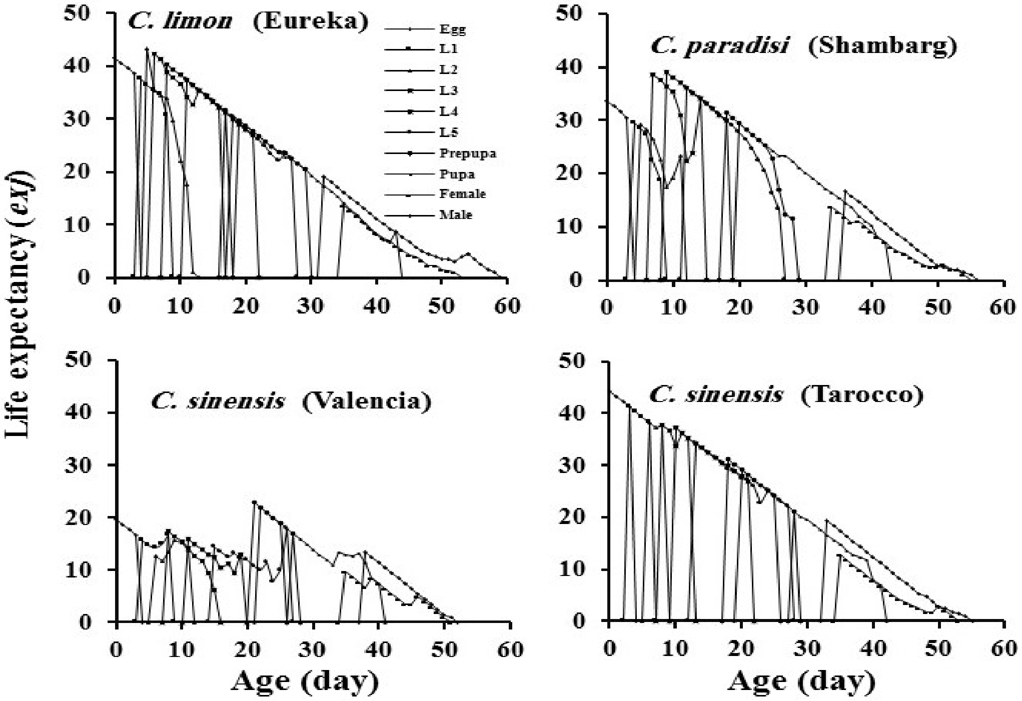

Figure 2 Age-specific life expectancy (e_{xj}) of *Papilio demoleus* on various citrus hosts at 24 ± 1 °C, 75 ± 5% RH and 16: 8 (L: D) h.

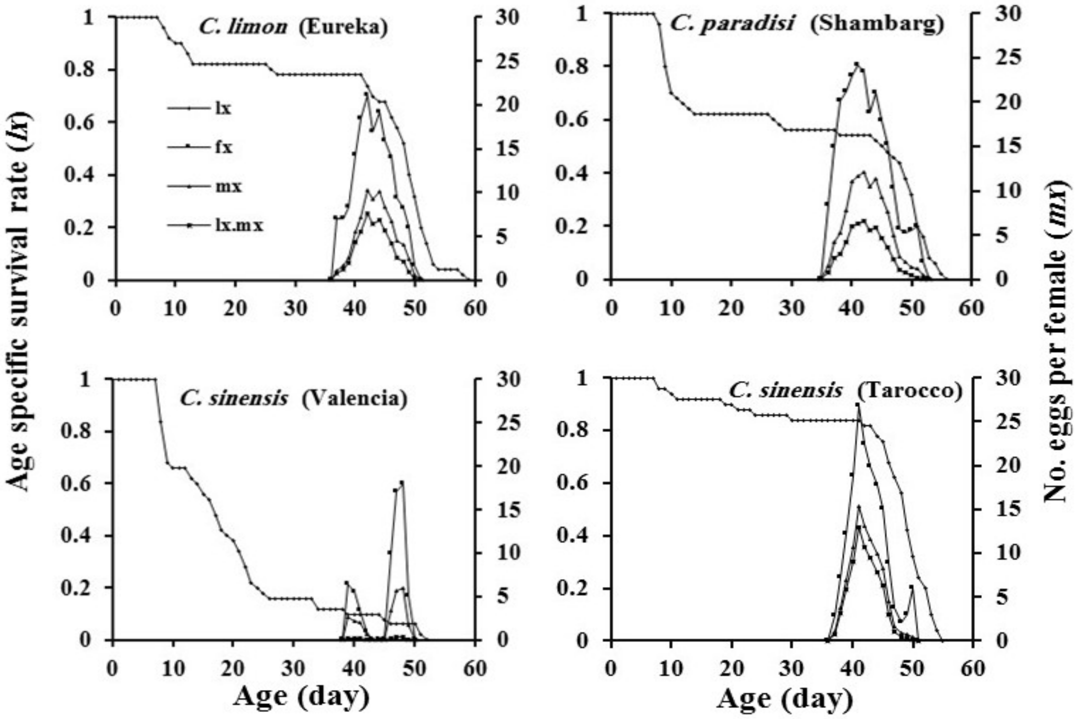

Figure 3 Age-specific survival rate (l_x),age-specific fecundity (m_x),female age-stage-specific fecundity (f_x), and age-specific maternity ($l_x m_x$) of *Papilio demoleus* reared various citrus hosts at 24 ± 1 °C, 75 ± 5% RH and 16:8 (L: D) h.

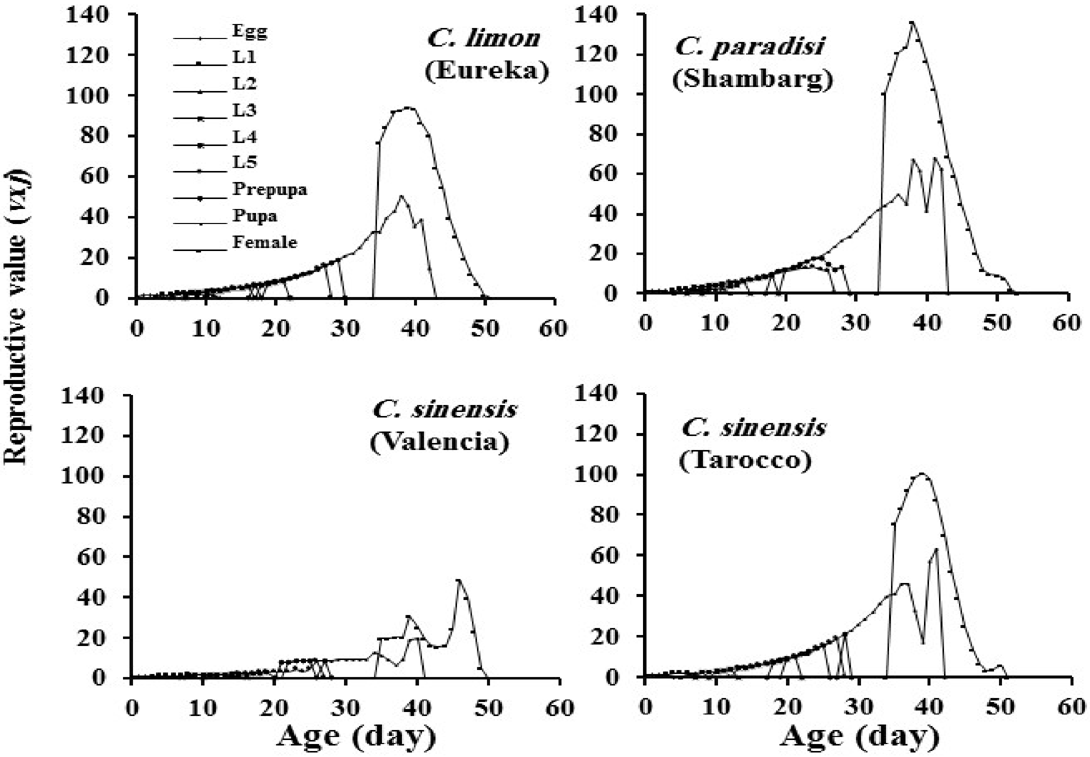

Figure 4 Age-specific reproductive value (v_{xj}) of *Papilio demoleus* on various citrus hosts at 24 ± 1 °C, 75 ± 5% RH and 16:8 (L: D).

Population growth parameters

The intrinsic rate of natural increase (r_m) varied from 0.011 to 0.099 (day $^{-1}$), which was lowest on *C. sinensis* (Valencia) and highest on *C. sinensis* (Tarocco).

The highest value of the finite rate of increase (λ) was on *C. sinensis* (Tarocco) and it is lowest on *C. sinensis* (Valencia).

The highest and lowest amounts of the gross reproductive rate (mean number of eggs per individual) of *P. demoleus* were observed on *C. sinensis* (Tarocco) and *C. sinensis* (Valencia) varieties (71.12 ± 11/024 and 1.99 ± 1/10) respectively. There were significant differences) P < *0.05* between the GRR on the four varieties. The highest and lowest amounts of the GRR of *P. demoleus* were observed on *C. sinensis* (Tarocco) and *C. sinensis* (Valencia) (88.9 ± 13.19 and 26.77 ± 15.45).

The longest and shortest mean generation times (T) were recorded on *C. limon* (43.85 days) and *C. sinensis* var. *valencia* (37.48 days).

Discussion

Looking for resistant varieties plays a key role in integrated pest management programs (Wilson and Huffaker, 1976; Endo *et al.*, 2007). In the present study differences among varieties could be attributed to differences in quality of components of host plant varieties (such as carbon, nitrogen, and defensive metabolites). Moreover, plant quality varies considerably depending on external environmental factors (such as predictable changes between seasons and less predictable changes initiated from environmental stresses) and these could be cited as other reasons for the difference (Awmack and Leather, 2002). Adult insects need high-carbohydrate food as their main source of energy for longevity, fecundity and mobility. The low number of eggs laid on a plant could have been impressed by the more indirect route of reduced fecundity arising from larva feeding on nutrient-poor plants (Verkerk and Wright, 1996; Hamilton *et al.*, 2005).

The results obtained in this study showed that the host plant significantly affected development, survival, and fecundity of the citrus butterfly. With regard to insect–plant interactions, it is useful to assess the impact of the different host plant varieties on the performance of herbivorous insects (Azidah and Sofian-Azirun, 2006; Saeed *et al.*, 2009).

The developmental time of immature stages of citrus butterfly was shorter when reared on *C. sinensis*, (Tarocco) and its survival rate was higher when reared on *C. paradisi* (Shambarg). The longevities of both female and male *P. demoleus* adults were not affected. Total developmental time was lowest on *C. sinensis* (Valencia) (9.67 ± 1.51) and was highest on *C. sinensis* (Tarocco) (12.38 ± 0.39), *C. paradisi* (Shambarg) (12.29 ± 0.57) and *C. limon* (Eureka) (12.09 ± 0.49). These parameters give an indication of population growth rate of an insect pest in the current and next generations (Frel *et al.*, 2003). The life table parameters, particularly, intrinsic rate of natural increase (r_m) is reflective of many factors such as fecundity, survival and generation time, and shows the potential of a population to increase under optimum conditions. It would also be a most appropriate index to evaluate the performance of an insect on different host plants (Kocourek *et al.*, 1994; Southwood and Henderson, 2000). The r_m value of *P. demoleus* ranged from 0.099 to 0.011 which was lowest on *C. sinensis* (Valencia) and highest on *C. sinensis* (Tarocco) (Table 2). The higher r_m value of *P. demoleus* on *C. sinensis* (Tarocco) may be due to the greater fecundity, lower mortality and shorter developmental time of the pest fed on this variety. Therefore, *P. demoleus* fed on *C. sinensis* (Tarocco) could have greater potential to increase population or have outbreaks in the next generation. However, lower r_m value on *C. sinensis* (Valencia) could be attributed to poor fecundity and survivorship of *P. demoleus* on this variety (Naseri *et al.*, 2009).

Generally, slow development or digestion and lower fertility in phytophagous insects are due to lower food quality (Chen *et al.*, 2004). To compensate for shortage of nutrients, insects acquire or process the food by behavioral (increased consumption) or physiological (increased digestion, absorption or conversion) response or a combination thereof (Slansky and Scriber, 1985; Simpson and Simpson 1990). Furthermore, in the field conditions, weak host plant quality may have an indirect effect on population density by increasing the exposure time of insects to their natural enemies as a result of long-term developmental times (Sarfraz *et al.*, 2006). There are many factors that can affect food suitability, including nutrient content. The production of chemicals such as toxins and digestibility reducers may interfere with the physiology of the insects and decrease survival (Schoonhoven *et al.*, 2005).

Table 2 Mean (± SE) population growth parameter of *Papilio demoleus* on four citrus hosts at 24 ± 1 °C, 75 ± 5% RH and 16:8 (L: D) h.

Population growth parameter	*Citrus limon* (Eureka)	*Citrus paradisi* (Shambarg)	*Citrus Sinensis* (Valencia)	*Citrus sinensis* (Tarocco)
The intrinsic rate of increase r_m (day $^{-1}$)	0.080 ± 0.005^a	0.092 ± 0.055^a	0.011 ± 0.012^b	0.099 ± 0.0038^a
The finite rate of increase λ (day $^{-1}$)	1.090 ± 0.0055^a	1.090 ± 0.0061^a	1.012 ± 0.0120^b	1.100 ± 0.0042^a
The net reproduction rate R_0 (offspring per individual)	49.50 ± 10.460^b	25.99 ± 19.750^b	1.99 ± 1.100^c	71.12 ± 11.024^a
GRR (Offspring per individual)	74.03 ± 14.12^a	53.81 ± 11.78^b	26.77 ± 15.45^c	88.9 ± 13.19^a
The mean generation time T (day)	43.85 ± 0.53^a	42.89 ± 0.63^a	37.48 ± 16.09^b	42.76 ± 0.34^a

Means in a row followed by the same letters are not significantly different at the 0.05 level (ANOVA).

Previous research on nutritional indices and amylolytic activities of *P. demoleus* reared on four varieties of citrus showed that the efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) values and amylolytic activities were lowest on *C. limon* (Eureka) (7.95 ± 0.32, 8.13 ± 0.27 and 0.08 ± 0.01), respectively and highest on *C. sinensis* (Tarocco) (26.21 ± 0.43, 39.05 ± 0.57 and 0.38 ± 0.01), respectively. (Kholghi Eshkalak *et al*., 2016). Which is in agreement with the results obtained in the present study regarding life table parameters. These results indicated that *C. limon* (Eureka) is unsuitable hosts for *P. demoleus.*

According to the results, the four closely related hosts had significant influence on life table parameters of *P. demoleus*. The pest showed a slightly better performance on *C. sinensis* (Tarocco) and *C. paradisi* (Shambarg) than on *C. sinensis* (Valencia) and *C. limon* (Eureka). Based on the present study it can be concluded that *P. demoleus* performs differently on the four tested hosts, providing some useful information that would promote understanding its statusas a pest on the hosts concerned. According to results obtained in this study, *C. sinensis* (Tarocco) and *C. paradisi* (Shambarg) appeared to be the most favorable hosts for *P. demoleus* among the varieties tested. Knowledge of how the quality of citrus varieties influences the biology of *P. demoleus* can help us understand the population dynamics and aid in management of this insect.

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تأثیر میزبان‌های مختلف مرکبات بر پارامترهای جدول زندگی پروانه‌ی برگخوار مرکبات *Papilio demoleus* (Lepidoptera: Papilionidae)

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چکیده: پروانه‌ی برگخوار مرکبات *Papilio demoleus* L.، یکی از آفات مهم مرکبات در بسیاری از نقاط جهان می‌باشد. در این پژوهش تأثیر چهار رقم تجاری مرکبات شامل گونه‌ی پرتقال (رقم پرتقال تاراکو و والنسیا)، لیموترش (رقم اورکا) و گریپ‌فروت (رقم شامبرگ) بر پارامترهای جدول زندگی و پارامترهای زیستی *P. demoleus* در شرایط دمایی ۱ ± ۲۴ درجه‌ی سلیسیوس، رطوبت نسبی ۵ ± ۷۵ درصد و دوره‌ی نوری ۱۶ ساعت روشنایی و ۸ ساعت تاریکی مورد مطالعه قرار گرفت. نتایج نشان داد که ارقام مختلف مرکبات بر طول دوره‌ی مراحل رشدی تأثیر معنی‌داری داشتند بیش‌ترین کل دوره‌ی رشد مراحل نابالغ (۰/۳۹ ± ۱۲/۳ روز) روی رقم تاراکو مشاهده شد. تفاوت پارامترهای جدول زندگی پروانه برگخوار مرکبات روی رقم‌های مختلف مرکبات معنی‌دار بود. بیش‌ترین و کم‌ترین نرخ متناهی افزایش جمعیت ۰/۰۰۴۲ ± ۱/۱۰ و ۰/۰۱۲ ± ۱/۱۲ به‌ترتیب روی ارقام تاراکو و والنسیا مشاهده شد و بیش‌ترین مقدار نرخ خالص تولیدمثل (R_0) (۱۱/۰۲۴ ± ۷۱/۱۲) روی رقم تاراکو به‌دست آمد. هم‌چنین بیش‌ترین نرخ ذاتی افزایش جمعیت (r) (۰/۰۰۳۸ ± ۰/۰۹۹روز) بود که روی رقم تاراکو به‌دست آمد و کم‌ترین مقدار آن (۰/۰۱۲ ± ۰/۰۱۱ روز) مربوط به رقم والنسیا بود. کوتاه‌ترین مدت زمان یک نسل (T) روی ارقام تاراکو و والنسیا مشاهده شد. نتایج به‌دست آمده از این پژوهش نشان می‌دهد که رقم تاراکو میزبان حساسی برای پروانه برگخوار مرکبات می‌باشد. نتایج این پژوهش و مطالعات مشابه در آینده روی میزبان‌های دیگر می‌تواند به‌عنوان پایه ای برای پیدا کردن میزبان‌های مقاوم در مدیریت این آفت درنظر گرفته شود.

واژگان کلیدی: برگخوار مرکبات، *Papilio demoleus*، جدول زندگی، پارامترهای رشد جمعیت، مرکبات